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ANALYSIS OF ESSENTIAL ELEMENTS AND ANTI FUNGAL ACTIVITY OF MEDICINAL PLANT *CASSIA OBVATA* COLLAD AGAINST DERMATOPHYTES

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Abstract

Different solvent extracts such as ethanol, methanol, ethylacetate, chloroform and aqueous extracts were obtained from leaves and shoot of a medicinal plant *Cassia obvata* Collad for screening the antifungal activity against dermatophytes such as *Aspergillus flavus, A. niger, Microsporum gypseum, Trichophyton tonsurans* and *T. rubrum* which were scraped from different skin portions of the patients. The aqueous extract showed maximum inhibition activity against test dermatophytes, as compared to ethanol, methanol, chloroform and ethylacetate extracts. Furthermore 9 elements such as Al, Ca, Cu, Fe, Mg, Mn. P, S and Zn were analysed from *Cassia obvata* Collad which have therapeutic role in the skin diseases.

Introduction

Cassia obvata Collad commonly known as Sanna is a traditional medicine in many parts of Asia for the treatment of various fungal skin diseases like *Tinea capitus, Tinea pedis, Tinea manum, Tinea corporis* etc. The fresh leaves and shoot of the plants are used for cooling and seed is used for headache, the leaves and fruit is used to adulterate, anthelmintic for intestinal worms and liver stimulant (Kritikar & Basu, 1935; Shahani & Memon, 1988; Baquar, 1989; Ansari *et al*., 1993; Sharma 2003; Pirzada & Abro, 2003).

Keratinolytic mycoflora love to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof, beak etc. They utilize keratin as carbon source (Cooke, 1980). Keratinophilic fungi are important ecologically and present in the environment with variable distribution patterns and cause human and animal mycoses (Mohamed *et al*., 2000). Most cutaneous infections are the work homogeneous group of keratinophilic fungi known as dermatophytes (Elewski, 1998). The dermatophytes have the capacity to invade keratinized tissue of the body including skin, hair and nails (Irene Weitzman & Summerbell, 1995).

Materials and Methods

Plant material: Plant material i.e., leaves and shoots of *Cassia obvata* Collad of the family Caesalpiniaceae were collected from different areas of Kohistan Region, District Dadu and identified through literature (Ali, 1973). The collected plant material was washed with distilled water and placed in shade at room temperature for two weeks. One kg of dried plant material was dipped in five litre of ethanol solvent in bottle for 20 days for cold percolation. The extract was filtered and concentrated under reduced pressure below 40^{o}C using rotary evaporator. The residue completely dried in powdered form. From the residue five different extracts i.e., ethanol, ethylacetate, chloroform, methanol and aqueous extracts were prepared using separating funnel. The extracts were left at

room temperature, so that the solvents completely evaporated and organic compounds remained in dry form. These compounds were mixed with sterilized water (1 g: 5 ml). Each extract was tested for antifungal activity.

Collection of dermatophytes: The dermatophytic fungi viz., *Aspergillus niger*, *Aspergillus flavus*, *Microsporeum gypseum*, *Tricophyton tonsurance* and *Tricophyton rubrum* were examined under normal light by removal of infected hair, skin and nails in the form of scales, crust and hair stumps. The infected hair, scalp and nail particles were inoculated on the slanted surface of Sabouraud dextrose agar (SDA) medium and incubated at 27-30ºC for up to three weeks. Species of the dermatophytes isolated from Civil Hospital Hyderabad & Department of Dermatology, Liaquat University Hospital, Jamshoro Sindh, during this study were identified on the basis of their growth characteristics and microscopic morphology (Nasreen *et al*., 2006).

Preparation of fungal culture: Sabourad glucose-agar medium was prepared by using the components: Pepton 10 g, Glucose 20 g, Agar 20 g, and distilled water, 1 litre with 5.4 pH. All the contents were mixed and dissolved in distilled water. The solution was autoclaved at 120^{o}C, 15 p.s.i., for 20 minutes.

Treatment of different solvent extract layers: The human skin pathogens were treated with different extracts and results were taken after 72 hours at 30^{o}C. (Usmanghani & Shameel, 1986).

Methodology for elements determination: Appropriate working standard solution of Aluminum (Al), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Sulphur (S), Zinc (Zn) were prepared from stock standard solution (1000 ppm) in 2N nitric acid. Calibration curves were drawn for each elementS using Atomic Absorption Spectrophotometer Hitachi model 180-50 and U.V. Spectrophotometer. The calibration curves obtained for concentration V.S, absorbance data were statistically analyzed using fitting of state line by least square method. A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

The efficiency of extraction method was checked by standard addition method. The sample was spiked with known standards and digested with nitric acid and hydrogen peroxide mixture. The matrix of standard and sample solution was same. The percentage recovery test for different elements by digestion method adopted was 98.5-99% in range.

Results

All the crude extracts showed significance antifungal activity against most of the fungi, but the activity of inhibition varied for the fungi with respect to the type of plant extracts used (Table 1).

Ethanol extract: The maximum inhibition activity was observed against *T. tonsurans* and *T. rubrum* (81.82% and 80%) while moderate inhibition activity was noticed against *M. gypseum* and *A. niger* (66.67% and 50%). It exhibited minimum inhibition activity against *A. flavus* (42.86%).

Table 1. Antifungal activity of different solvent extract of *Cassia obvata* against test organisms.

Test extract	*A. niger*	*A. flavus*	*T. tonsurans*	*M. gypseum*	*T. rubrum*
Ethanol					
Control reading at 30°C after 72 hours (mm)	40.0	35.0	55.0	30.0	25.0
Inhibited reading at 30°C after 72 hours (mm)	20.0	20.0	10.0	10.0	05.0
Inhibited (%)	50.0	42.86	81.82	66.67	80.0
Methanol					
Control reading at 30°C after 72 hours (mm)	40.0	35.0	55.0	30.0	25.0
Inhibited reading at 30°C after 72 hours (mm)	10.0	15.0	10.0	10.0	05.0
Inhibited (%)	75.0	57.15	81.82	66.67	80.0
Chloroform					
Control reading at 30°C after 72 hours (mm)	40.0	35.0	55.0	30.0	25.0
Inhibited reading at 30°C after 72 hours (mm)	15.0	14.0	15.0	10.0	05.0
Inhibited (%)	62.5	60.01	72.73	66.67	80.0
Ethyl acetate					
Control reading at 30°C after 72 hours (mm)	40.0	35.0	55.0	30.0	25.0
Inhibited reading at 30°C after 72 hours (mm)	14.0	15.0	15.0	10.0	05.0
Inhibited (%)	65.0	57.15	72.73	66.67	80.0
Aqueous					
Control reading at 30°C after 72 hours (mm)	40.0	35.0	55.0	30.0	25.0
Inhibited reading at 30°C after 72 hours (mm)	10.0	15.0	155.0	05.0	03.0
Inhibited (%)	75.0	57.15	72.73	83.34	88.0

Methanol extract: The maximum inhibition activity was observed against *T. tonsurans* and *T. rubrum* (81.82 and 80% respectively). While moderate inhibition activity was noticed against *A. niger* and *M. gypseum* (75 and 66.67%) and minimum inhibition activity against *A. flavus* (57.15).

Chloroform extract: The maximum inhibition activity was observed against *T. rubrum* 80% while moderate inhibition activity was noticed against *T. tonsurans, T. rubrum* and *A. niger* (72.73, 66.67 & 62.5% respectively) and minimum inhibition activity against *A. flavus* (60%).

Ethylacetate extract: The maximum inhibition activity was observed against *T. rubrum* and *T. tonsurans* (80%, 72.73% respectively), while moderate inhibition activity against *M. gypseum* and *A. niger (*66.67 and 65%) and minimum inhibition activity against *A. flavus* (57.15%).

Aqueous extract: The maximum inhibition activity was observed against *T. rubrum* and *M. gypseum* (88 and 83.34% respectively), while moderate inhibition activity was found against *A. niger* and *T. tonsurans* (75 and 72.73%) and minimum inhibition activity against *A. flavus* (57.15%).

The considerable amount of elements such as Aluminum (Al), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Sulphur (S) and Zinc (Zn) have been determined from the medicinal plant *Cassia obvata* Collad (Table 2). These elements are biologically very important for the treatment of different skin diseases.

Discussion

In the present study crude extracts of the plant material obtained in polar and less polar organic solvents were tested against fungi causing skin diseases.

Table 2. Quantity of different elements in *Cassia obovata* Collad.

Name of elements	Formula	Amount mg/ kg
Aluminum	Al	4.39-4.90
Calcium	Ca	11683.98-13909.05
Copper	Cu	9.69-11.82
Iron	Fe	89.02-104.55
Magnesium	Mg	3934.15-4255.94
Manganese	Mn	31.22-35.87
Phosphorus	P	133.33-156.03
Sulphur	S	824.97-836.50
Zinc	Zb	33.74-47.84

All the crude extracts showed significant antifungal activity on most of the fungi, aquous extract had maximum inhibition activity (57.15-88%) against test organisms as compared to aqueous, chloroform, methanol and ethyl acetate. The Methanol extract had very active inhibition activity (57.15-80%) against test dermatophytes, while ethanol chloroform and ethyl acetate extracts had moderate inhibition activity (42.86-80%) against test organisms.

In the present study first attempt was made to investigate the antifungal activity of the medicinal plant *Cassia obvata* Collad against dermatophytic Fungi such as *A. niger, A. flavus, T. tonsurans, M. gypseum* and *T. rubrum*. The solvent extracts were very active against test organisms except ethanol extract, which was very weak against *A. niger* and *A. flavus*. Furthermore nine essential elements such as Al (4.39-4.90) mg/kg, Ca (11683.98–13909.5) mg/kg, Cu (9.68-11.82) mg/kg, Fe (89.0-104.55) mg/kg, Mg (3943.15-4255.94) mg/kg, Mn (31.22-35.87) mg/kg, P (133.33-156.03) mg/kg, S mg/kg and Zn (33.74-47.84) mg/kg have been analyzed, which play some therapeutic role against different skin diseases (Janjua, 1990; Saily *et al*., 1994, Sahito *et al*., 2003).

The current experiments provide some scientific justification for the utilization of crude extract of *Cassia obvata* Collad for the treatment of different skin diseases such as *Tinea capitus, Tinea corporis, Tinea manum, Tinea pedis* etc.

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